INTRASPLENIC XENOGRAFTING OF HUMAN FETAL PANCREATIC ISLET CELL CULTURES INTO RATS WITH EXPERIMENTAL DIABETES MELLITUS

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Injection of cultures of human fetal pancreatic islet cells (PIC) into the liver of rats (through the portal vein or directly into the parenchyma) caused a lasting reduction of the blood sugar to the normal or nearly normal level and clinical remission of the diabetic state throughout the period of observation [2, 5, 6].

This paper gives the results of experiments on xenografting human fetal PIC cultures into the splenic pulp of rats with alloxan diabetes.

EXPERIMENTAL METHOD

PIC cultures were prepared from the pancreas of cadavers of human fetuses aged 5-6 months by the method described previously [1, 4]. The cells were cultured in medium 199 with 10% bovine serum in 100-ml and 200-ml flasks. Separate foci of growth were fixed with 1% glutaraldehydein 0.2 M cacodylate buffer (pH 7.2-7.4), postfixed with 1% OsO_4 solution, and embedded in Araldite M. Ultrathin sections cut on an LKB-8800 microtome were stained with uranyl acetate and lead citrate. Sections were studied and photographed in the JEM-100B electron microscope (magnification 5000-50,000).

Experiments were carried out on noninbred male albino rats weighing 220-260 g. Experimental diabetes mellitus was produced by subcutaneous injection of alloxan in a dose of 200 mg/kg body weight. Rats in which the blood sugar exceeded 350 mg% for not less than 2 weeks after injection of alloxan were used subsequently. The glucose concentration in the blood and urine was determined by the orthotoluidine method. The rats were weighed twice a week. The animals were divided into three groups: 1) eight normal rats not receiving alloxan; 2) eight rats with untreated alloxan diabetes; 3) eight rats with alloxan diabetes treated by transplantation of human fetal PIC cultures into the splenic pulp. A 5-8-day culture obtained from one pancreas was used for one transplantation. Laparotomy was performed on the recipient rats and cultured PIC were injected into the splenic pulp from a syringe through an ordinary injection needle or plastic catheter. The volume of cell suspension injected was 0.5-0.8 cm^3. The site of injection was covered with MK-2 glue. No immunodepression was used. Splenectomy was performed on two rats of group 3, 10 days after transplantation. The spleen removed from these animals was studied histologically.

EXPERIMENTAL RESULTS

Electron-microscopic study of foci of a 5-day culture obtained from the pancreas of a 22-week human fetus showed that the ultrastructure of the epithelial cells composing them corresponded to the typical ultrastructure of B cells of islets of Langerhans, as described in the literature [9].

B granules in various stages of formation were found in their cytoplasm. Early granules had average electron density and were homogeneous. In the next stage of development the contents of the granules were separated from the membrane by a narrow electron-translucent annular zone. Granules were formed and accumulated in immediate contact with membranes of the

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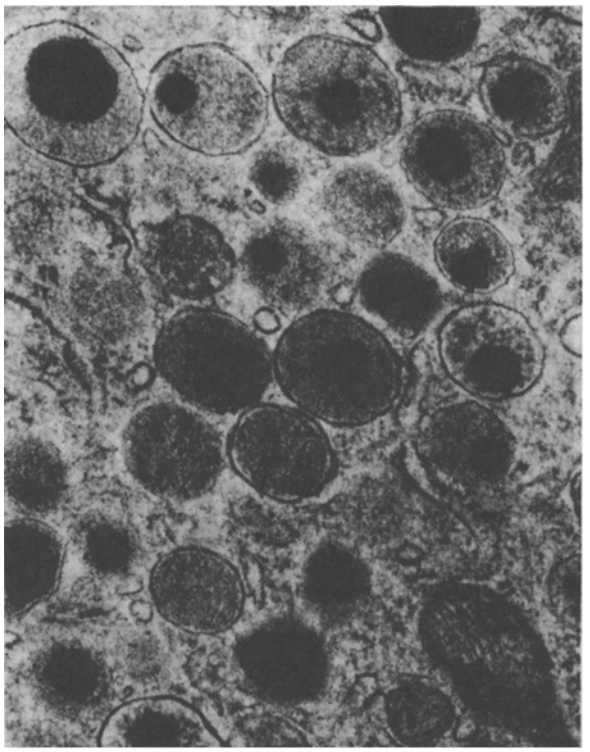

Fig. 1

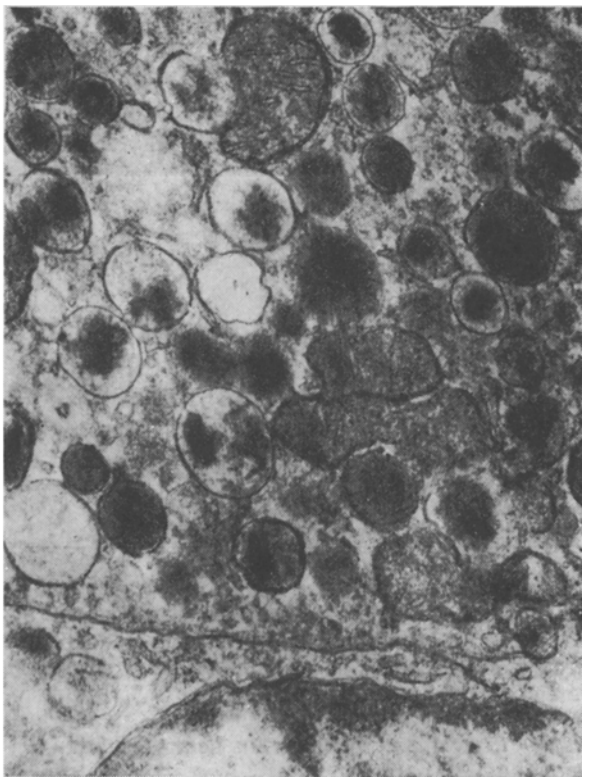

Fig. 2

Fig. 1. Area of cytoplasm of pancreatic B cell in 22-week human fetus. B granules in early stages of development. Here and in Fig. 2, 5th day of culture, 40,000 ×.

Fig. 2. Pancreatic B cell of 22-week human fetus. B granules in late stages of development.

Golgi complex (Fig. 1). In the next stage an electron-dense core, a middle zone with average electron density, and a peripheral "halo" with low electron density could be distinguished in the B granules. In the most mature granules the electron-dense mass was shifted and situated eccentrically and the middle zone acquired a floccular structure (Fig. 2). The peripheral electron-translucent zone of such granules was much wider and lost its regular outlines. The diameter of the B granules varied from 180 to 350 nm. Granule accumulation predominated in some cells, discharge of secretion in others.

In the experimental rats hyperglycemia and glucosuria developed as early as 1-2 days after injection of alloxan. The animals developed apathy, polydipsia, polyphagia, and polyuria, their hair began to fall out, and they lost considerable weight. Spontaneous reversal of diabetes did not take place in any of the rats of group 2; their hyperglycemia (over 385 mg%) and glucosuria (over 2.8%) continued throughout the period of observation. The body weight of these animals was less than that of the rats of groups 1 and 3.

In six of the eight rats of group 3 a marked fall in the glucose concentration in the blood and urine took place 5-7 days after transplantation of PIC. After another 7 days, normoglycemia and aglucosuria were observed in three rats. In the remaining three animals lower levels of hyperglycemia (165-190 mg%) and glucosuria (0.3-0.8%) were observed throughout the period of observation (up to 4 months). Correction of the blood sugar and reduction of the glucosuria were accompanied by disappearance of clinical signs of diabetes; by the end of the 1st week after transplantation the animals ceased to gain weight, in the same way as the rats of group 1. Splenectomy was performed on two rats with normoglycemia 8 weeks after the onset of a stable remission of diabetes. In these animals a marked rise of the glycemia was observed 1 week after splenectomy — to 285 and 230 mg%, respectively (Fig. 3). The hyperglycemia, which continued thereafter, was accompanied by recurrence of the clinical features of diabetes. Histological examination of the removed spleen showed numerous large and small clusters of PIC in the red pulp. Some PIC were in various stages of destruction (Fig. 4).

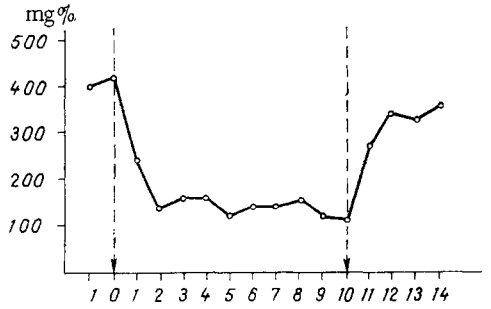

Fig. 3. Changes in blood sugar of rats with alloxan diabetes after intrasplenic transplantation (arrow on left) of 6-day culture of PIC of a 24-week human fetus followed by splenectomy (arrow on right). Abscissa, blood sugar; ordinate, weeks.

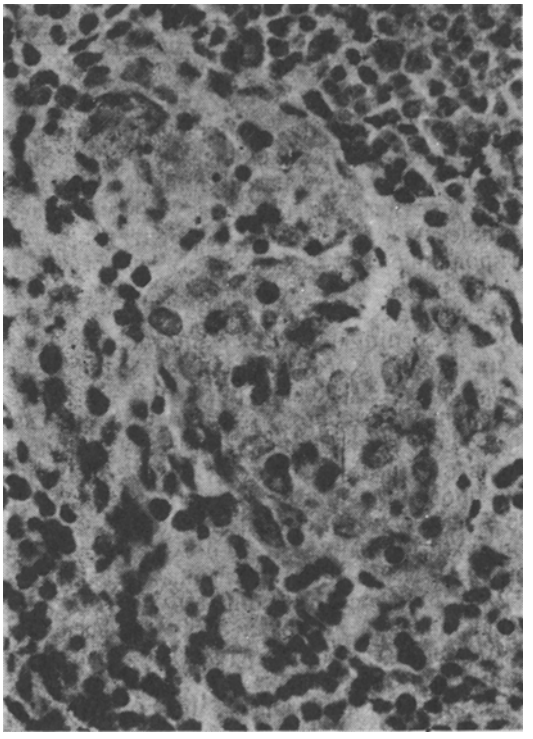

Fig. 4. Group of PIC in splenic pulp (10 weeks after xenografting). Some cells in a state of destruction. Hematoxylin–eosin, 400 ×.

Electron-microscopic investigations showed that foci of growth of cultures obtained from the human fetal pancreas consist mainly of B cells of the islets of Langerhans. It was shown previously that cultures of this kind actively produce insulin [3]. Transplantation of cultures of PIC from human fetuses into the spleen of rats with alloxan diabetes had a long-lasting antidiabetic action. Recurrence of the hyperglycemia in the two rats of group 3 after splenectomy indicates that the remission of diabetes in these animals was due to the functional activity of PIC transplanted into their spleen. This is confirmed by the results of histological investigation of the removed spleen.

The beneficial effect of transplantation of PIC into the splenic pulp of animals with alloxan and streptosotocin diabetes has been reported in experimental studies of iso- and allografting of islets of Langerhans [7, 8]. Our experiments showed that intrasplenic xenografting of cultures of human fetal PIC into rats with alloxan diabetes is equally effective to intrahepatic transplantation of these cultures.

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